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RESEARCH ARTICLE

Preparation of modified magnetic nanoparticles for in vitro delivery of ceftriaxone

Arash Alipour*, Mohammad Saber Tehrani, Parviz Aberoomand Azar, Mohammad Hadi Givianrad

Department of Chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran

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ABSTRACT

The present study introduces a new approach for the surface radical grafting of n-vinylcaprolactam as a thermoresponsive agent and 3-allyloxy-1,2-propandiol with an affinity toward ceftriaxone onto modified Fe_3O_4 nanoparticles by 3-mercaptopropyltrimethoxysilane. Characterization of the grafted nanoparticles was performed by Fourier Transform Infrared Spectroscopy, Elemental Analysis, and Vibrating Sample Magnetometer. The surface morphology was examined by Scanning Electron Microscopy. The obtained grafted nanoparticles were employed to determine trace ceftriaxone in biological human fluids. Ceftriaxone uptake profile by the modified magnetic nanoparticles demonstrated that the active sites were well accessible in the grafted nano carrier. Results showed that the adsorption behavior was fittable by the Langmuir adsorption isotherm model. Moreover, our investigation included solid phase extraction for simulated human biological fluids (e.g., gastric and intestinal fluids). Recovery of extraction of human biological fluids was over 90% was achieved in the present research. Overall, magnetic nanoparticles coated with sensitive polymer have proved to enjoy unique properties in biotechnology and drug delivery research.

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INTRODUCTION

Ceftriaxone (CFX), marketed with the brand Rocephin, is an advantageous antibiotic to treat multiple bacterial infections. The conditions are middle ear infections, pneumonia, meningitis, bone, endocarditis, skin infections, gonorrhea, and pelvic inflammatory disease. Occasionally, the drug has preoperative uses and after a bite wound in order to avoid infection [1, 2]. Ceftriaxone is administrable via injecting intravenously or into a muscle. Pain at the injection site and allergic reactions are among the typical fallouts. Further likely fallouts are C. difficile-related diarrhea, hemolytic anemia, gall bladder disease, and seizures. This drug is not indicated in patients with a previous history of anaphylaxis to penicillin but has likely uses in individuals who have presented slighter reactions. Provision of the endogenous form is not allowed together with endogenous calcium. Existing provisionary proofs suggest ceftriaxone to have a relative safety in the course of gestation and breastfeeding. This drug is a third-generation cephalosporin working through prevention of bacterial cell wall synthesis. The commonly-used procedures of ceftriaxone examination in biological specimens are on the basis of High-Performance Liquid Chromatography analysis with UV detector (HPLC-UV) method [3-5], Ultraviolet-Visible spectroscopy (UV/Vis) [6-9], Gas Chromatography-mass spectrometry (GC-MS) [10], Tandem Quadrupole (Triple Quadrupole) Mass Spectrometry (LC–MS/MS) [11], differential-pulse adsorptive stripping voltammetry [12] as well as TLC [13,14], and capillary electrophoresis [15,16]. Magnetic nanoparticles (MNPs) have been developed as drug carriers in the past decade owing to their fine nanostructure and nanoscale particle size. MNPs have gained a widespread ground in magnetic-targeting systems for drug releasing, particularly in

* Corresponding Author Email: *arash.alipour@srbiau.ac.ir*

cancer treatment. Surface modification via grafting organic polymer chains is a beneficial approach to promote the application of nanoparticles, which is applicable for biomedical uses. The co-precipitation method was applied herein to synthesize magnetic nanoparticles. In addition, the free-radical graft copolymerization NVC/AP modified magnetic nanoparticles surfaces is reported with 3-mercaptopropyltrimethoxysilane. The present research aimed at developing a procedure for extracting ceftriaxone from biological human fluids by the presented polymer-grafted magnetic nanoparticle as a sorbent. As controlled drug release is a substantial topic of interest, the manufactured nano-sorbent was applied for drug delivery [17-21]. An alternate method was employed for functionalization of magnetic nanoparticles by NVC/AP to interact with ceftriaxone for controlled drug release during a protracted duration in reaction to temperature fluctuations.

EXPERIMENTAL

Chemicals and reagents

All the reagents and chemical substances underwent no additional purification and treatment and were directly utilized in here. These include 3-mercaptopropyltrimethoxysilane, tetraethylortosilicate, trifluoroacetic acid (TFA), n-vinylcaprolactam (NVC), 3-Allyloxy-1,2-propanediol, ethanol, acetic acid, methanol (HPLC gradient grade), and 2,2'-azobisisobutyronitrile (AIBN). The whole substances and other products were procured from Merck Company (Darmstadt city, Germany http://www.merck-chemicals.com/). Double distilled water (DDW) was used for dilution procedures.

Instruments

Records of Ultra Violet-Visible (UV/Vis) spectra were taken by the Perkin Elmer/Lambda 25 UV/Vis spectrophotometer (USA). Infrared spectra were determined by a Jasco Fourier transform infrared spectrometer (FT-IR-410, Jasco Inc., and Easton, Maryland, USA). Elemental analysis (CHN) was conducted using a Thermo-Finnegan (Milan. Italy) model Flash EA elemental analyzer. The morphology of the MNPs was characterized with Scanning Electron Microscopy (SEM, EM 3200, KYKY Corporation, China) and the magnetic properties of the nanoparticles were evaluated via a vibrating Sample magnetometer (VSM, Homade 2 tesla), where a magnet (25°c, 17.50 × 20 mm, 5500 Oe) was used for collecting the MNPs. The pH was quantified by a metrohm meter, model 744 (Zofingen, Switzerland).

Chromatographic conditions

The chromatographic separation was done on Dionex Acclaim 120 C18 (100x4.6 i.d) mm, 5µm, stainless steel column. The mobile phase including a mixture of acetonitrile, potassium phosphate buffer, and trimethylamine with 10:90:0.2 ratio, (pH 7.0) was supplied at a flow rate of 1.0 mL/min. The mobile phase was passed through 0.45µm membrane filter and degassed by sonication before usage. Separation was implemented at room temperature and detection was carried out at 240 nm.

Synthesis of absorbent

Four steps of absorbent synthesis were as follows: synthesizing MNPs, coating MNPs using Tetraethylorthosilicate, modification of MNPs with 3-mercaptopropyltrimethoxysilane, and ultimately polymer grafting.

Synthesis of MNPs

The co-precipitation method used in the present study involved synthesizing MNPs via 2:1 of Fe (II) and Fe (III) together with ammonium solution under N_2(g) atmosphere. In brief, 3.97 g Fe(II).$4H_2O$ and 2.307 g Fe(III).$6H_2O$ were dissolved in 100 ml light water. Afterward, isolation of the solution was done in nitrogen medium wherein ammonium solution was instilled gently. The solution underwent strong mixing at 82˚C for 120 min. Finally, the obtained dark brown precipitates were gathered by a magnet and rinsed with light water several times to attain the neutral pH [22].

Coating MNPs using TEOS

The MNPs synthesized in the preliminary step were relocated to a container followed by adding 40 ml of Tetra Ethyl Ortho Silicate, 82 ml of ethanol, and 2 ml of ammonium to set the pH at 11. The mixture was then shaken swiftly for 2 days. This solution was washed twice with light water. Separation by magnet was done and precipitates were dried out in vacuum desiccators [23,24].

Modification of MNPs

The mixture comprising 47.5 mL of dry Toluene, 2.5 mL of 3-mercaptopropyltrimethoxysilane, and

2 g of manufactured MNPs was poured into a bottle and vortexed at 90˚C for 20 minutes. Next, the bottle content was rinsed by normal toluene and dried in a vacuum desiccator [25,26].

Polymer grafting

Primarily, 40 mL of ethanol, 4 mL of 3-allyloxy-1,2-propanediol, 2 g of n-vinylcaprolactam, and 0.25 g of 2,2'-azobisisobutyronitrile trigger were poured into the bottle and dissolved in DDW. Thereafter, 2 g of modified Fe_3O_4 nanoparticles from the previous stage was added and stirred while being insulated under N_2(g) atmosphere at 75˚C for 8 hours. Finally, the mixture was rinsed with 22 ml of ethanol and dried out in vacuum desiccator.

Batch method

Here, 1.5 mL drug solution, containing 1 mg/L ceftriaxone were made to which 0.02 g MNPs@[NVC-co-3-AP] were added while pH was adjusted to 6 by Britton–Robinson buffer solution. Next, the mixture was shaken for 10 minutes. Fe_3O_4@[NVC][AP] was separated by a magnet, after which the supernatant solution was decanted. To encourage desorption of drug from the surface of Fe_3O_4@[NVC][AP], methanol, as an elution solvent, was added which was measured by HPLC at 260 nm.

RESULTS AND DISCUSSION

Characterization of nanoparticles

Characterization of the polymer grafted magnetic nanoparticles was performed by elemental analysis, Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and Vibrating-sample magnetometer (VSM). FT-IR spectra indicated that grafting was achieved successfully. The peaks at 3535.57 cm^{-1} and 561.22 cm^{-1} belonged to O—H stretching and Fe—O stretching bands, respectively. The FT-IR spectrum of modified MNPs verified that C—H and S—H groups were present at 2920.72 cm^{-1} and 2190.78 cm^{-1}, respectively. The peaks of O—H and C—O in Fe_3O_4@[NVC][AP] established that the nano carrier was grafted to the MNPs. Table 1 summarizes the datum of the elemental analysis. For Fe_3O_4@[NVC][AP], negligible alterations were noticed in C, H, and N percentages, in comparison to raw MNPs. The surface morphology of Fe_3O_4@[NVC][AP] was were using SEM. The SEM image of Fe_3O_4@[NVC][AP] reveals that spherical agglomerated nanoparticles were present having a diameter of below 50 nm and also determines the ununiformed porous surface (Fig. 1). The prepared product was examined by VSM testing as well. The saturation magnetization (Ms) was detected to be 70.37 emu/g and 50.16 emu/g for pure MNPs and Fe_3O_4@[NVC][AP], respectively. This denoted that magnetic properties of particles following coating with a large saturation magnetization (Ms) resulted in a rapid and easy separation of the MNPs/polymer from the reaction medium in the magnetic field. Such an observation indicates strongly that the two samples (MNPs and Fe_3O_4@[NVC][AP]) display superparamagnetic behavior. The outcomes of Ms and Mr are highly important in the applications of the magnetic targeting carriers and biomedical fields (Fig. 2).

Table 1. Result of elemental analysis

elemental analysis	C%	H%	N%
magnetic nanoparticles	86	0.08	0.04
Fe_3O_4@[NVC][AP]	4.11	1.21	0.89

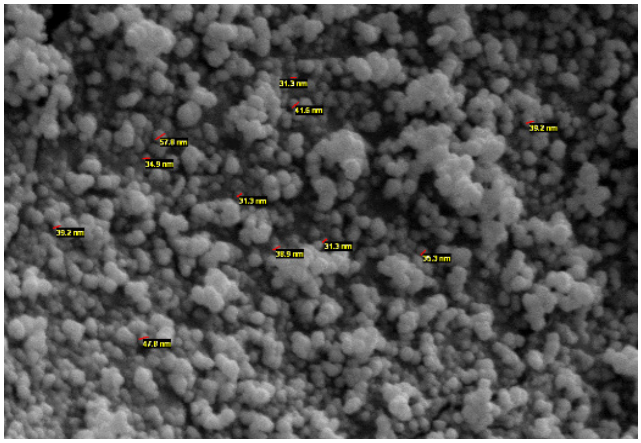

Fig.1. SEM image of Fe_3O_4@[NVC][AP]

Refinement of adsorption and desorption of ceftriaxone by the synthesized polymer

In order to achieve greatest efficiency of adsorption and delivery of drug by the synthesized Fe_3O_4@[NVC][AP] in the laboratory, a range parameters including pH, time of adsorption, temperature of adsorption and release, capacity of polymer, and adsorption isotherm were taken into account.

Investigation the optimal pH

Investigation of the optimal pH was performed to obtain the maximum level of adsorption by Fe_3O_4@[NVC][AP]. Study of synthesized nanoparticles with ceftriaxone was carried out at different pHs. In this research, the adsorption of ceftriaxone by Fe_3O_4@[NVC][AP] was monitored at different pHs, which affected this absorption. In order to discover the optimal pH, the pH range 2-9 was investigated with preferable pH found through the following equation:

$$Q = (C_0 - C_e)V\ /W \quad (1)$$

$$C_e = (20A_{sample}) / A_{Standard} \quad (2)$$

In the above formula, Q is the valence of adsorption (mg/L), V represents the solution volume (L), C_e and C_0 are final concentration (mg/L) and initial concentration (mg/L), respectively, and W is the absorbent weight (g). The results obtained for each pH are reported in Fig. 3, where the optimal pH for adsorption of ceftriaxone is pH=8.

Optimizing of sorption temperature

The impact of time was investigated on the efficiency of the drug adsorption by the synthesized polymer. In order to discover the time required for reaching the maximum adsorption of drug on the Fe_3O_4@[NVC][AP] nanoparticles, durations of 1 to 60 minutes were studied. In early 15 minutes, the maximum adsorption of drug by the synthesized polymer was clearly observed. Over time, a constant portion of adsorption was reached. The results obtained from examining the impact of time are reported in Fig. 4. According to the results,

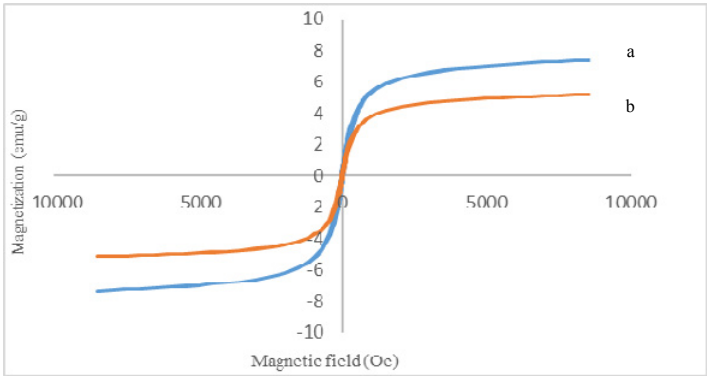

Fig. 2. Magnetization hysteresis loop behavior of Fe_3O_4 (a), Fe_3O_4@[NVC][AP] (b)

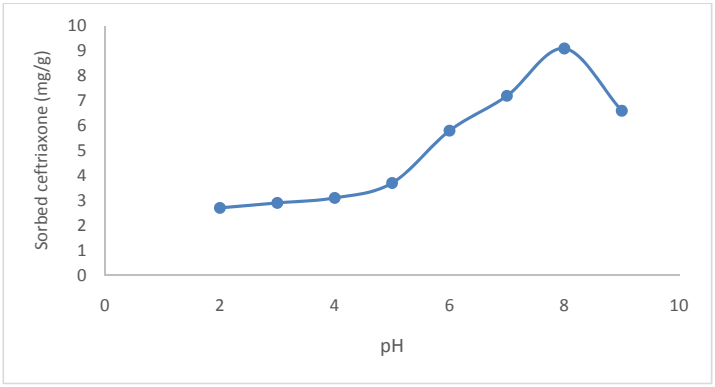

Fig. 3. pH effects on sorption of ceftriaxone onto Fe_3O_4@[NVC][AP]

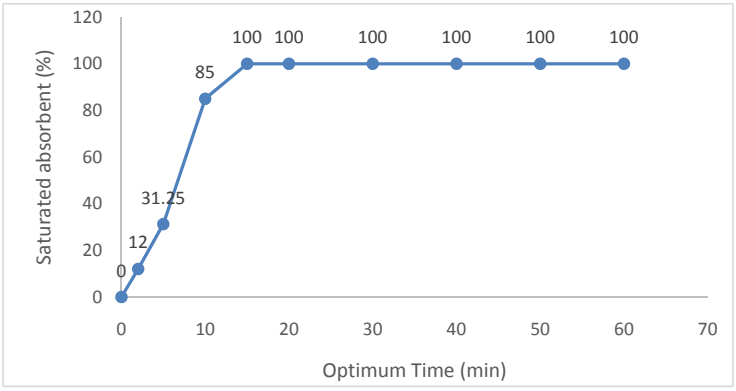

Fig. 4. Kinetics of ceftriaxone onto Fe_3O_4@[NVC][AP]

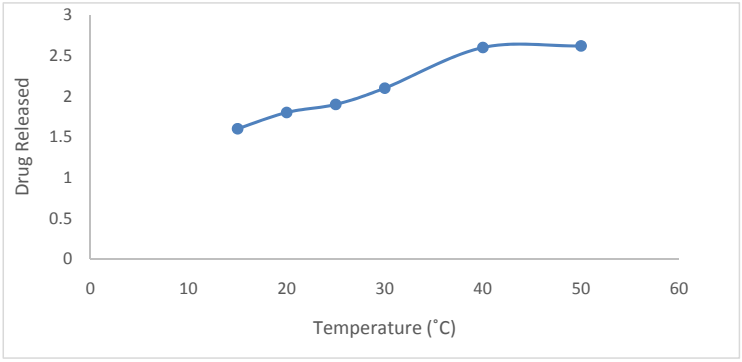

Fig. 5. Impact of temperature on releasing of ceftriaxone by Fe_3O_4@[NVC][AP]

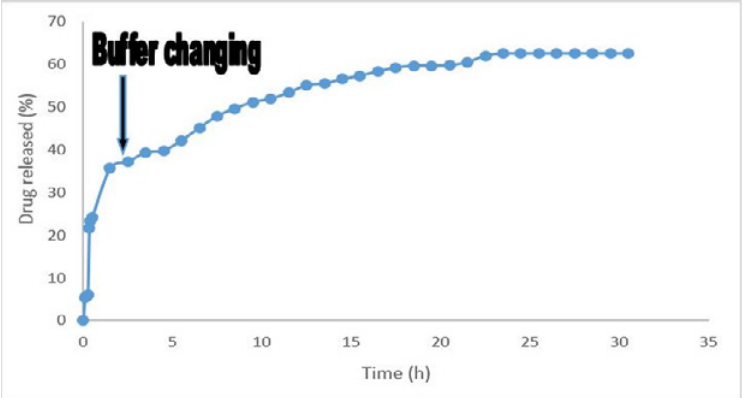

Fig. 6. Ceftriaxone delivery profile in simulated gastric and intestinal fluids

the maximum drug adsorption by the synthesized polymer was reached within the minimum time possible. Indeed, very good kinetics were observed by the adsorption process hence no long residual time was necessarily required for the polymer in the drug solution.

Release of drug was researched within the temperature range of 15 to 50˚C. Due to sensitivity of the polymer to temperature, release of drug was peaked with elevation of temperature to 40˚C as in Fig. 5. On the other hand, no change in amount of desorption was observed at temperatures higher

than 40˚C; therefore, 40˚C can be considered as the optimal release temperature.

Determination of capacity and adsorption isotherm of synthesized polymer to adsorb ceftriaxone

Since the extent of adsorption is a function of temperature, either increase or decrease in temperature causes altered adsorption capacity of the polymer. So, it is crucial to keep temperature constant to capture the polymer's capacity. At this stage, ceftriaxone solution (1-60 mg/l) containing 1 ml of optimum solution buffer at pH 8 and synthesized nano carrier at standard laboratory temperature (25˚C) was prepared to examine the capacity of the produced polymer.

The outcomes gained regarding the study of polymer's capacity are studied. The polymer has been able to absorb ceftriaxone drug in a wide range of concentrations. The capacity of 60 mg/l of the synthesized polymer for adsorption of ceftriaxone was 1.24 mg/g polymer. Based on Langmuir equation, this number has reached its maximum, 1.24 mg/g, so each gram of the synthesized polymer can exclusively absorb 60 mg/l of ceftriaxone.

$$1/q_0 = 1/\left(K_l\, q_{max}\right)\, 1/C_0 + 1/q_{max} \qquad (3)$$

The results obtained on the capacity of the synthesized polymer for adsorption of drug at 20˚C were investigated by Langmuir model [27,31].

In vitro drug release

Both of human biological fluids (gastric fluid pH 1.2, intestinal fluid pH 7.4) were simulated to test the release course of the drug from Fe_3O_4@[NVC][AP]. Beakers containing the drug loaded on Fe_3O_4@[NVC][AP] were shaken, 30 rpm, at 37˚C. Specific time interludes were set to take samples. Then, HPLC instrument was used to determine the drug content of each sample (Fig. 6).

Kinetic drug release

The delivery procedure of ceftriaxone on Fe_3O_4@[NVC][AP] was simulated in both of human biological fluids (gastric and intestinal fluids).

In the early 30 min at simulated body temperature, since severe acidic condition dominates in the stomach, 38% of ceftriaxone was approximately released in gastric media with a sharp slope, though a ceftriaxone release of 60% occurred in simulated intestine with a smooth slope up to 10 h at 37˚C.

CONCLUSION

The successful synthesis of a new nano carrier grafted magnetic nanoparticles was reported as an effective and appropriate sorbent for extracting ceftriaxone. A superb ceftriaxone sorption rate was obtained on Fe_3O_4@[NVC][AP]. Following extraction, trace ceftriaxone in biological human fluids was determined by the HPLC method. The observations indicated that the developed approach is significantly advantageous, which include convenience, effectiveness, and excellent stability for analyzing ceftriaxone. Eventually, it is hoped that greater number of investigators with an interdisciplinary knowledge to work on magnetic-targeted drug delivery systems to broaden the scope of this field.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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